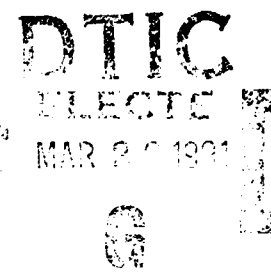


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ROLE OF MONONUCLEAR PHAGOCYTES IN THE PATHOGENESIS OF HUMAN IMMUNODEFICIENCY VIRUS INFECTION¹

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MONONUCLEAR PHAGOCYTES AS A RESERVOIR FOR VIRUS IN THE HIV-INFECTED PATIENT

Infection by the human immunodeficiency virus (HIV) initiates a slowly progressive degenerative disease of the immune system, termed the *acquired immunodeficiency syndrome* (AIDS). The primary immunologic defect in AIDS is an inexorable depletion of CD4⁺ T cells, a depletion invariably associated with opportunistic infection, degenerative neurologic disease, a variety of neoplastic changes, and ultimately death (1). Attempts to detect viral protein or nucleic acids in blood leukocytes of seropositive patients reveal a frequency of infected cells of no more than 0.001% (2). Paradoxically, this low frequency of infected cells remains constant from onset of infection through late-stage disease. There is now solid evidence, however, that consideration of the low numbers of infected cells in blood alone grossly underestimates the viral load in the HIV-infected patient. In

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certain bodily tissues, such as those of the central nervous system, lymph nodes, or lung, the frequency of HIV-infected cells may be 10,000- to 100,000-fold higher than that in blood. In each of these tissues, the predominant cell type infected with HIV is not the CD4+ T cell, but rather the macrophage.

Neurologic disease associated with HIV infection is characterized by typical pathologic changes in brain and spinal cord. HIV-induced changes in the brain are most evident within the white matter, and they include gross cerebral atrophy without inflammation but associated with microglial nodules (clusters of microglia and reactive fibrous astrocytes) and multinucleated giant cells. Virus isolation from cerebrospinal fluid or homogenates of brain tissue is successful in most patients with AIDS-associated encephalopathy (3). Indeed, virus isolation from cerebrospinal fluid of patients with acute aseptic meningoencephalitis during HIV infection can occur before seroconversion (4). Southern blot analysis of DNA from cerebral cortex detects HIV sequences at a relative abundance equal to or greater than that from spleen, liver, lymph nodes, or lung (5). In situ hybridization for HIV RNA in brain tissue of infected individuals shows a frequency of productively infected cells of 1-10%. The predominant infected cell (and in most studies, the only infected cell) is the macrophage (6, 7). Brain macrophages (subarachnoid, perivascular, and parenchymal cells), microglia, and macrophage-derived, multinucleated giant cells possess 500 to 1500 copies of HIV RNA per cell. This amount of virus RNA per infected cell is at least 10-fold higher than that found in blood leukocytes (2). It is interesting that almost all of the HIV-infected brain macrophages are negative for CD4 by immunocytochemistry (8).

HIV-induced pathologic changes in the spinal cord are different from those in brain. Vacuolar myelopathy with macrophage infiltrates is found at autopsy in about 25% of patients with AIDS (9). The high frequency of HIV-infected macrophages with myelin in phagocytic vacuoles suggests a proximate role for these cells in the pathogenesis of AIDS-associated myelopathy syndrome.

Transmission electron microscopic analysis of lymph nodes from HIV-infected individuals showed typical virions in virtually all specimens examined (26 of 30 lymph nodes), even in those patients with early asymptomatic infection (10, 11). Viral particles are found only in follicular dendritic cells, with an approximate frequency of 10% (12). Similarly, HIV can be isolated from cells in bronchoalveolar lavage fluids (about 50% macrophages) (13). HIV proteins or nucleic acids are detected in 10-50% of the macrophages in such fluids (14, 15). Epidermal Langerhans cells, the dendritic, CD4+ antigen-presenting cells of skin, are also targets for HIV infection. In skin biopsies of 40 seropositive patients, HIV-infected Langerhans cells were

identified in about 20%. However, such HIV-infected Langerhans cells were present at a frequency of infection much lower than that for macrophages of brain, lymph node, or lung (16). Indeed, other investigators found immunocytochemical evidence for HIV infection in cells of the oral mucosa in only 2 of 26 seropositive patients (17) or in 0 of 44 skin biopsies (18). Taken together, these studies suggest that while Langerhans cells are susceptible targets for HIV infection (19), the frequency of this event during HIV disease is probably closer to that of the CD4⁺ T cell in blood (0.001%) than to that of other tissue macrophages.

CHANGES IN MONONUCLEAR PHAGOCYTE NUMBER, PHENOTYPE, OR FUNCTION DURING HIV INFECTION

Numerical, phenotypical, or functional changes that occur in the various macrophage subpopulations during HIV infection are not well defined. Observations made by different investigators using similar and often identical experimental techniques often conflict. Moreover, virtually all studies to date suffer two major interpretive problems. First, patient selection has been limited to individuals with late-stage, symptomatic HIV infection. The average time from seroconversion for HIV antibody to onset of disease may be 10 yr (1). Analysis of macrophage numbers, morphology, phenotype, or function in this time period is almost nonexistent. Second, the macrophage subpopulation most frequently analyzed has been the blood monocyte. This precursor cell to all tissue macrophages has a relatively short circulating half-life in blood of about 30 hr. Migration of blood monocytes into tissue is unidirectional: Unlike T-cell traffic patterns, there is no evidence for tissue macrophage reentry into the blood. Most blood monocytes examined for any change during HIV disease are not infected. As previously stated, the frequency of HIV-infected cells in blood (monocytes and lymphocytes) is not more than 0.001%. Thus even major changes in such an infected cell population are below the limits of detection for virtually all assays.

Most studies (but not all) document normal numbers of blood monocytes in HIV-infected patients even during late-stage disease when CD4⁺ T cells may be undetectable (20–22). Similarly, most studies document normal phenotypic expression of plasma membrane antigens by flow cytometric analysis with monoclonal antibodies. Expression of the class-II major histocompatibility complex (MHC) determinants (HLA-DP, HLA-DQ, HLA-DR) by monocytes from HIV-seropositive individuals is indistinguishable from that of cells from seronegative donors (23). Changes in class-II MHC determinants induced in monocytes after treatment *in vitro*

with interferon γ (IFN γ) or with bacterial endotoxic lipopolysaccharides were similar to those induced in control cells (24). Expression of several other plasma membrane determinants (CD4, CD11, CD14, CR3, transferrin receptor, Fc receptor I and II, or Mo3e) by monocytes from HIV-infected patients was also normal (25). Still other studies performed with similar or identical methodologies document significant changes in the expression of these monocyte membrane antigens (26–28). While it is difficult to reconcile such disparate observations, recent reports that the envelope glycoproteins of HIV, gp41 and gp120, both act directly on monocytes to induce phenotypic or functional change suggest a mechanism that may explain such variability (29–32). Indeed, that expression of HLA-DR on monocytes was decreased only in those patients with detectable levels of p24 capsid protein in their blood supports this hypothesis (33).

Analysis of macrophage function during HIV infection has been approached both in vivo and in vitro. Several studies by Bender and colleagues show impaired clearance from circulation of particles that express Fc (spleen macrophage-mediated clearance) or C3 (hepatic Kupffer cell-mediated clearance) determinants in most patients with late-stage HIV infection. Interestingly, defective Fc-mediated particle clearance was observed in early HIV disease only in those patients with immune thrombocytopenic purpura (34). In vitro assays that assess monocyte function suffer interpretive problems identical to those previously mentioned for phenotypic changes. Monocyte chemotactic responses to any of several different chemoattractants are each depressed below normal levels (20, 35). This phenomenon can be duplicated with monocytes from seronegative donors after exposure of cells to purified gp41 or gp120 proteins (29, 32). Monocyte microbicidal activity against any of several unrelated pathogens (*Candida albicans*, *C. guilliermondi*, *C. neoformans*, *Aspergillus fumigatus*, *Thermoascus crustaceus*, *Toxoplasma gondii*, *Chlamydia psittaci*) is normal both in steady state and after further in vitro exposure to IFN γ (36–38). Phagocytosis of latex beads or infectious microbes such as *Candida* or *Toxoplasma* is normal (20, 36, 40). Release of toxic monocyte secretory products that serve as effector molecules in antimicrobial reactions such as H₂O₂, interleukin-1 (IL-1), or tumor necrosis factor (TNF α), was normal with cells from HIV-infected donors and each appropriately increased after further in vitro treatment with IFN γ or with bacterial endotoxic lipopolysaccharides (23, 36, 37, 39, 41). Moreover, monocytes from HIV-infected patients treated with recombinant IFN γ in vivo also showed increased secretion of H₂O₂ and microbicidal activity against *T. gondii* (39).

Examination of tissue macrophages in HIV-infected patients suggests a different picture from that of the relatively normal circulating blood mono-

cyte population. In skin, Langerhans cells undergo extensive morphologic change even in early disease. Up to 30% of epidermal Langerhans cells show condensation of cytoplasmic and nuclear chromatin, vacuole formation, and cytolysis in the absence of obvious HIV infection (16). Detection of HIV virions by transmission electron microscopy or of HIV proteins by immunocytochemistry were rare events. Other studies show profound phenotypic changes in epidermal Langerhans cells in otherwise unaffected, clinically normal skin: The numbers of cells that express HLA-DR or CD1 or show ATPase activity decrease to at least 50% of control levels with late-stage HIV disease (42-44).

Follicular dendritic cells in lymph nodes also show major degenerative changes early in HIV infection; these changes increase in extent with disease progression (10-12). Indeed, in late-stage disease there can be complete loss of the follicular dendritic network so important for antigen presentation in lymph nodes (45). Unlike observations with the epidermal Langerhans cell, viral particles are easily detected in follicular dendritic cells (11, 45). Interestingly, Langerhans cells of lymph nodes also do not show HIV virions even in close proximity to obviously infected follicular dendritic cells. The degenerative changes observed in epidermal Langerhans cell and lymph node follicular dendritic cell populations may represent a special event not apparent with macrophages of other tissues. Unlike other tissue macrophages, the Langerhans cell and the follicular dendritic cell have exceedingly high levels of expression of cell-membrane CD4. Mechanisms (largely unknown) that induce depletion of CD4+ T cells with time after HIV infection may also affect the Langerhans and follicular dendritic cells. Blood monocytes and tissue macrophages have low to undetectable levels of CD4 and therefore may not be susceptible to these degenerative or lytic events.

MONONUCLEAR PHAGOCYTES AS SUSCEPTIBLE TARGET CELLS FOR HIV IN VITRO

Initial attempts to infect blood monocytes or alveolar macrophages with HIV suggested productive infection, but the results were inconclusive. Monocytes cultured with HTLV-III_B, a strain of HIV passaged continuously in T cells or T-cell lines, bound virus to the cell membrane and ingested viral particles into phagocytic vacuoles within 10 min. HIV was detected in such vacuoles by transmission electron microscopy through 3 days of culture, but no virions were observed budding from the plasma membrane. Assays for reverse transcriptase activity, p24 antigen, or other viral proteins by direct immunofluorescence were uniformly negative (46). Addition of mitogen-induced lymphoblasts from seronegative donors to

such HIV-infected monocyte cultures 2-3 weeks after the initial virus exposure initiated a productive infection in the T-cell targets. Thus, at the very least, monocytes were able to sequester viable HIV and to transmit these infectious particles to T cells (47). Other studies with HTLV-III_B and cultured monocytes showed low levels of reverse transcriptase activity (twice background) 2 weeks after virus inoculation, but no cytopathic effects were induced in the cell monolayer and no cell-associated virions were observed by transmission electron microscopy. The frequency of HIV-infected cells in these cultures as quantified by direct immunofluorescence for HIV proteins was 1-5% (48).

Not surprisingly, the first evidence for productive HIV infection of monocytes in vitro developed from studies using primary cultures of brain tissue from patients with AIDS-associated encephalopathy (49). Primary brain explants, enriched for macrophages by repeated trypsin digestion of adherent cell monolayers, released reverse transcriptase activity through 6 weeks of culture. Virus budding from plasma membranes of cultured cells was evident by transmission electron microscopy in a small fraction of cells. This relatively low number of infected cells after 6 weeks of culture was confirmed by in situ hybridization for HIV RNA. Cocultivation of blood monocytes from seronegative donors with the cell-free culture fluids of macrophage-enriched, HIV-infected explants of brain tissue initiated a productive infection sustained in the monocyte target cells through at least 2 months. Such monocytes infected with HIV showed a frequency of infected cells in culture of 5-20% by immunofluorescence with monoclonal anti-p17 capsid protein and developed profound HIV-associated cytopathic effects of multinucleated giant cells not present in the uninfected control cultures. Serial dilutions of virus inoculum derived from the brain explant cultures were 10- to 100-fold more efficient for infection of other monocyte target cells than for T cells. Conversely, HTLV-III_B was 10,000-fold more efficient in infection of T-cell than monocyte targets.

Such distinct differences in target cell tropism for different HIV isolates were confirmed in a subsequent study (50). HIV isolated from cerebrospinal fluid of a patient with AIDS-associated encephalopathy replicated in PHA-induced lymphoblasts from seronegative donors, but not in blood monocyte cultures. In contrast, virus isolated from brain tissue infected both lymphoblasts and monocyte target cells. Restriction endonuclease cleavage maps of these two HIV strains were different at only 4 of 26 restriction enzyme sites (15%), a difference much less than that found between two isolates from different patients (15 of 33 sites, or 45%). Thus, biologically different but closely related strains of HIV with distinct target cell tropism coexist in various tissues within the same infected patient.

Culture of Human Monocytes

Detailed analysis of HIV-monocyte interaction was impeded by the inability to culture blood monocytes for extended intervals in vitro. Conventional culture of monocytes as an adherent monolayer in medium with fetal calf serum results in death of most of the initial cell population within 7 days (80% loss of viable cells in 1 week). In contrast, monocytes cultured in medium with human serum and recombinant human macrophage colony stimulating factor (MCSF) survived for weeks with little or no loss of cell viability (51). MCSF in murine macrophage cultures induces a strong proliferative response and colony formation in agar. In contrast, for human macrophage cultures, MCSF is a survival and differentiation factor, not a growth factor (52). Low levels of ^3H -thymidine incorporation are observed in such cultures, but the numbers of proliferating cells as quantified by counting cells with mitotic figures or nuclear grains on ^3H -thymidine autoradiography were 1–3% of the total cell population. Phenotype analysis of this monocyte population at 2 weeks in culture documented a cell population >98% monocytes. The CD4 determinant, the HIV receptor for T cells, was undetected in this cell population by any of several different monoclonal antibodies. Although CD4 is present at low concentrations in blood monocytes, the number of cells that display this plasma membrane determinant has been reported to range from 5% to 90% (23, 49, 51, 53–55). Indeed, several groups fail to detect CD4 by flow cytometric analysis in monocytes, or they detect it on <5% of total cells (51, 55).

Repeated attempts to infect MCSF-treated monocytes with HTLV-III_B were uniformly negative even with viral inoculum 100,000-fold higher than that necessary to infect T cells. In contrast, virus isolation onto MCSF-treated monocytes from blood leukocytes of HIV-infected patients was much more successful. Peripheral blood mononuclear cells (PBMC) from 33 individuals seropositive for HIV or at risk for HIV infection were cocultivated with MCSF-treated monocytes from seronegative donors (H. E. Gendelman, L. Baca, H. Husayni, J. M. Orenstein, J. A. Turpin, D. Skillman, D. L. Hoover, M. S. Meltzer, "Macrophage-human immunodeficiency virus interaction: viral isolation and target cell tropism," submitted for publication). Culture fluids were assayed at 2- to 3-day intervals for p24 antigen by ELISA (Table 1). Significant levels of p24 antigen were detected in 31 to 33 cultures, an overall viral isolation frequency of 93%. HIV was detected in cultures of MCSF-treated monocyte target cells with PBMC from patients independent of the subject's age, sex, numbers of CD4⁺ T cells, or clinical stage. This relatively high frequency of virus isolation was also unaffected by coincident 3'-azido-3'-deoxythymidine

Table 1 Virus isolation from blood leukocytes of patients at risk for or infected with HIV

WR stage ^a	Serotype	Number of patients	Patient leukocytes added to:	
			MCSF-treated monocytes	PHA/IL-2-treated lymphoblasts
0	neg	2	2/2	0/2
1/2	pos	10	8/10	2/9
3/4	pos	7	7/7	2/3
5/6	pos	14	14/14	9/14
Total		33	31/33 (93%)	14/28 (50%)

^a Walter Reed staging classification for HIV infection (85): stage 0 (seronegative individuals with known risk factor); stage 1/2 (seropositive minimally symptomatic patients); stage 3-4 (T-cell depletion without opportunistic infection); stage 5, 6 (frank AIDS).

therapy. The average time interval to first detect p24 antigen in culture fluids was 20 ± 2 days (mean \pm SEM for 25 patients) with a median time of 18 days (range of 7 to 45 days). The time necessary to first detect p24 antigen in cultures with PBMC of patients with normal numbers of CD4+ T cells (830 ± 160 cells/mm³) and early disease was significantly longer than that of patients with decreased numbers of CD4+ T cells (160 ± 20 cells/mm³) and later stages of disease: 29 ± 5 days for 7 early-stage patients vs 17 ± 2 days for 17 later-stage patients.

The ability to isolate virus from PBMC in mitogen-stimulated lymphoblast cultures (the conventional T-cell isolation of HIV) increases with each stage of HIV disease. Virus isolation is successful in about 20-30% of seropositive, asymptomatic patients, and increases in frequency to about 80% in patients with frank AIDS. Thus, unlike the virus isolation in T cells, recovery of HIV on MCSF-treated monocytes is equally successful in both early and late stages of disease. Indeed, several reports show that recovery of HIV on macrophages may be the only effective virus isolation system in early disease: in a seropositive patient with laboratory-acquired HIV infection or in several seronegative patients with acute HIV infection, virus was isolated from blood only in cultured monocytes, and not in T cells (56, 57).

HIV isolates from patient PBMC in MCSF-treated monocytes were serially passaged in MCSF-treated monocyte cultures. Passage was successful with 17 of 20 isolates, an efficiency of 84%. The average time interval necessary to first detect p24 antigen in these cultures was 7 ± 1 days (mean \pm SEM for 16 isolates) with a median time of 7 days (range of 2 to 19 days). HIV-associated cytopathic changes in monocyte monolayers (multinucleated giant cells, cell syncytia, and lysis in about 20-40% of the cell population) were apparent at 2 weeks in all cultures (Figure 1). There

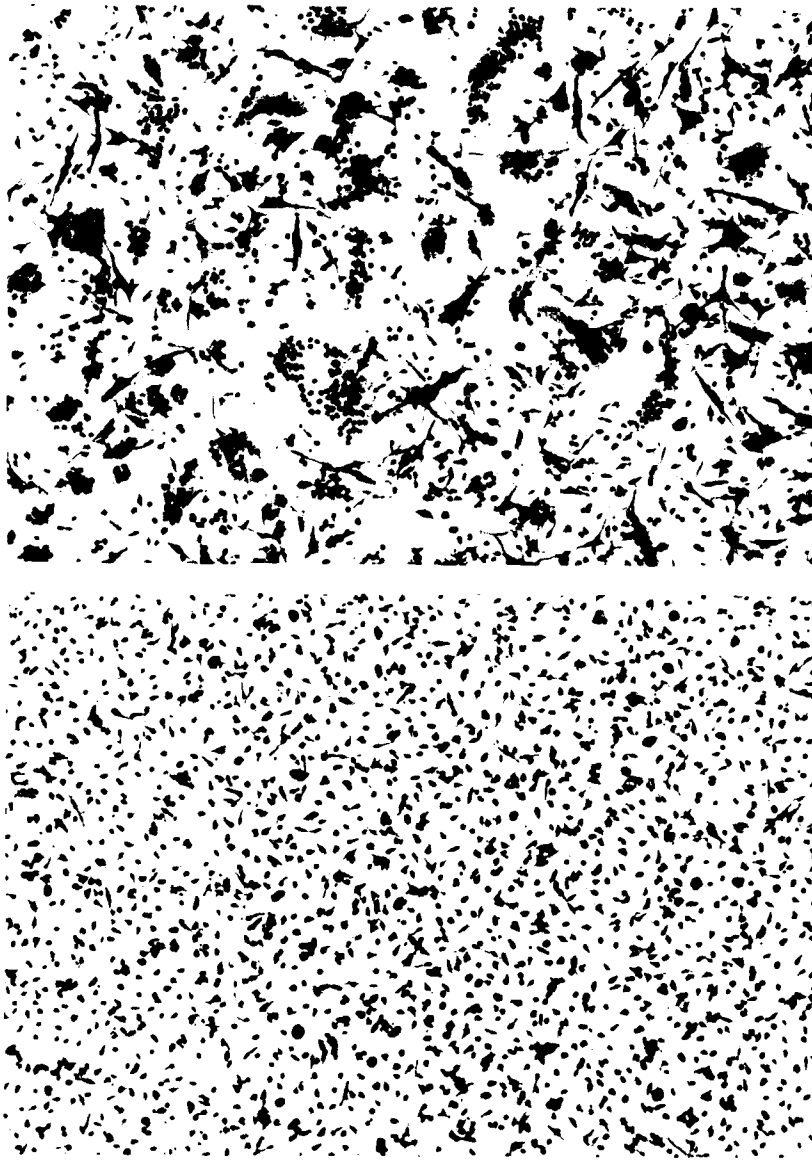


Figure 1 HIV-1 induced cytopathic effects in M-CSF-treated monocytes. PBMC from seronegative donors purified to >98% monocytes were cultured as adherent monolayers in medium with 10% human serum and 1000 U/ml MCSF. At 10 days, macrophages were exposed to 10^3 TCID₅₀ ADA, a monocyte tropic HIV-1 isolate. Cultures were refed with fresh medium every 2 to 3 days. Photomicrographs of adherent cells 15 days after infection are at $200\times$ original magnification: (left) uninfected M-CSF-treated monocytes; (right) HIV-1 infected monocytes.

was no correlation between these cytopathic effects and the clinical stage at which the virus was isolated.

Cellular Tropism

When HIV isolates that had been serially passaged three times in MCSF-treated macrophages were added to PHA/IL-2 treated lymphoblasts, levels of p24 antigen released into culture fluids were indistinguishable from those of HTLV-III_B-infected lymphoblasts through 2 weeks of infection. Analysis of such HIV-infected lymphoblasts for levels of reverse transcriptase activity, by in situ hybridization for HIV-specific RNA (5–20% frequency of cells expressing HIV-specific mRNA), by formation of cell syncytia during infection (3–10% of total cells), by downmodulation of T-cell plasma membrane CD4 (60% CD4+ PHA/IL-2 treated lymphoblasts prior to infection vs 10% CD4 cells 1 week after infection), and by transmission electron microscopy (progeny virions budding at the plasma membrane only with no intracytoplasmic accumulation of viral particles) showed no qualitative or quantitative differences between HTLV-III_B and the MCSF-treated monocyte-derived HIV isolates. These experiments document little or no target-cell restriction in virus replication for HIV isolated from patient PBMC into MCSF-treated macrophages; viral isolates grew equally well in macrophages or PHA/IL-2-treated lymphoblasts.

The preceding observations contrast with previous reports of target-cell restriction in the propagation of HIV isolates (49–51). It is possible that target-cell permissiveness to HIV infection may vary with different viral isolates. To clarify this point, we examined the serial passage of five clinical isolates of HIV in both MCSF-treated monocytes and PHA/IL-2 treated lymphoblasts (Figure 2). PBMC from five different patients seropositive for HIV were cocultivated with both MCSF-treated monocytes and PHA/IL-2-treated lymphoblasts from seronegative donors. In each of the five patients, an HIV primary isolate was recovered in both monocyte and lymphoblast culture systems. For each of the five patients, HIV isolated in PHA/IL-2 treated lymphoblasts or MCSF-treated monocytes were serially passaged into cultures of the homologous cell type. Furthermore, HIV isolated in MCSF-treated monocytes also infected PHA/IL-2 treated lymphoblasts. In marked contrast, none of five viral isolates recovered from PHA/IL-2-treated lymphoblasts showed growth in the heterologous MCSF-treated monocytes by the criteria of p24 antigen release, reverse transcriptase levels, or infectious titer. Moreover, viral isolates initially recovered in MCSF-treated monocytes and then passaged in PHA/IL-2 treated lymphoblasts showed little or no evidence of virus growth when placed back into MCSF-treated monocytes. The preceding experiments

		T (10 ⁻³)			T (10 ⁻³)
	T	T (10 ⁻³)		T	T (10 ⁻³)
		M NO GROWTH			M NO GROWTH
		M NO GROWTH			M NO GROWTH
230		T (10 ⁻²)	429		T (10 ⁻³)
	T (10 ⁻²)			T (10 ⁻³)	
M		M NO GROWTH	M		M NO GROWTH
	M (10 ⁻¹)			M (10 ⁻²)	
		T (10 ⁻³)			T (10 ⁻⁴)
	T	T (10 ⁻²)		T	T (10 ⁻⁴)
		M NO GROWTH			M NO GROWTH
		M NO GROWTH			M NO GROWTH
350		T (10 ⁻⁴)	418		T (10 ⁻³)
	T (10 ⁻⁴)			T (10 ⁻³)	
M		M (10 ⁻¹)	M		M (10 ⁻¹)
	M (10 ⁻³)			M (10 ⁻²)	
		T (10 ⁻³)			
	T	T (10 ⁻³)			
		M NO GROWTH			
		M NO GROWTH			
359		T (10 ⁻³)			
	T (10 ⁻³)				
M		M NO GROWTH			
	M (10 ⁻³)				

Figure 2 Serial passage of HIV isolated from PBMC of patients seropositive or at risk for HIV infection in MCSF-treated monocytes and PHA-IL-2 treated lymphoblasts from seronegative donors. Culture fluids from HIV-infected MCSF-treated monocytes or PHA/IL-2 treated lymphoblasts (primary isolates, first and second passages of patient isolates 230, 350, 359, 418, and 429) were added to 10-day MCSF-treated monocyte or 3-day PHA-IL-2 treated lymphoblast cultures. After a 2 hr virus adsorption interval, cultures were washed and refed with fresh medium then and every 2 to 3 days through 2 months. HIV infection in monocyte and lymphoblast cultures was estimated through 2 serial passages by infectious titer (TCID₅₀) and p24 antigen in culture fluids.

document the existence of two distinct species of HIV: Viruses isolated in MCSF-treated monocytes show dual tropism and infect monocytes and T cells equally; viruses isolated in PHA/IL-2-treated lymphoblasts replicate only in T cells.

Target cell selection of diverse HIV variants may underlie the phenomenon of macrophage tropism. Virus was initially isolated from HIV-infected patient PBMC (about 10–15% monocytes) by cocultivation with MCSF-treated monocytes from seronegative donors. This initial isolate may be a mixture of HIV variants. Some variants replicate in monocytes from either the HIV-infected patient or the uninfected monocyte target cells. These variants (the "MT" viruses) infect either monocytes or T cells. Other HIV variants replicate only in the residual T cells from the infected patient (transmission electron microscopy shows a small number of viable T cells in these initial cultures through 40 days). These variants (the "T" viruses) infect only other T cells. Serial passage of this mixed virus inoculum (MT viruses and T viruses) onto purified (>98% monocytes), uninfected MCSF-treated monocytes selects those HIV variants able to infect monocytes (MT viruses). Since no T cells are offered as target cells, the T viruses are eliminated. In contrast, serial passage of this mixed inoculum onto uninfected PHA/IL-2-treated lymphoblasts selects only those HIV variants able to infect T cells. The progeny virus of this infection infect only T cells (T viruses). In this scenario, one must postulate some selective advantage for the T viruses in T cells such that the MT virus variants are lost. Given a choice, T cells preferentially replicate the T viruses over the MT virus variants.

Loss of capacity to infect macrophages (by the T viruses) may reflect any of several changes in the virus, the target cell, or their interaction such as: (a) mutational changes in virus structural or regulatory genes, (b) changes in processing of virus gene products at the transcriptional (either different types or relative amounts of viral proteins) or posttranscriptional (changes in virion-associated carbohydrate or lipid moieties) levels, (c) complementation of the gene products of different integrated viruses yielding a virus competent to infect macrophages (in the case of macrophage-derived HIV) or a virus that interferes with macrophage infection (in the case of T cell-derived HIV), or (d) host cell regulatory factors permissive of or restrictive to viral replication at any stage of the virus life cycle. While little evidence presently supports or refutes any of these alternatives, the phenomenon of distinct HIV variants with different target cell tropism is found both in vitro and in vivo (49–51).

The maximum numbers of monocytes infected with HIV in vitro as quantitated by in situ hybridization for HIV RNA is at least three-fold greater than that in lymphoblast cultures (60–90% vs 5–15%). But the

number of virions released into culture fluids of HIV-infected monocytes, as quantified by infectious titer or reverse transcriptase activity, is 10- to 100-fold less than that of infected lymphoblasts. The basis of this apparent dissociation between high numbers of infected cells and low levels of infectious virus released into the culture is revealed by transmission electron microscopic analysis. HIV-infected T cells show hundreds of viral particles associated with the plasma membrane: HIV assembles and buds only from the plasma membrane of infected T cells, and there is no intracellular accumulation of mature or even immature virions. HIV interaction with macrophages is quite different from that of T cells (Figure 3). Ultrastructural analysis of HIV-infected macrophages 2-6 weeks after infection (time intervals where 60-90% of cells express both HIV-specific mRNA and proteins) shows few or no virions at the plasma membrane. Yet these infected cells contain large numbers of viral particles which are localized almost exclusively to intracellular vacuoles. Infected macrophages display numerous vacuolar structures, unassociated with the plasma membrane; each of these contains scores of mature and immature virions. Indeed, HIV not only accumulates within these intracellular vacuoles but also assembles and buds from the vacuolar membranes. Morphologic evidence strongly suggests that these vacuoles are derived from the Golgi complex (58). In essence, the macrophage handles HIV virus much like any other secretory glycoprotein. HIV is assembled in the Golgi complex and transported in Golgi complex-derived vacuoles toward the plasma membrane. Significantly, the final step of secretion, exocytosis into the extracellular milieu, appears suppressed. The amount of virus released from HIV-infected macrophages, quantitated by reverse transcriptase or p24 antigen in culture fluids, is 10-fold less than that released by an equal number of infected T cells. Thus, the HIV-infected macrophage represents a veritable virus factory, but a factory whose entire output remains hidden from the host. Experiments confirm that the intracellular virions of HIV-infected macrophages are infectious. Release of these viral particles by freeze-thaw cycles increases the infectious titer of the culture fluids at least 10-fold (51). These *in vitro* observations have been confirmed in the AIDS patient. Macrophages in the brain of a seropositive individual also show intracellular localization of virus particles within vacuoles, and little or no virus was detected at the plasma membrane (58). Such virus, sequestered from host immunity within cytoplasmic vacuoles, represent a reservoir for continued infection. Release of infectious virus from this reservoir and dissemination of HIV into other macrophages or T cells could be initiated by any agent that perturbs macrophage function: factors released during inflammation, normal tissue remodeling, or host response to intercurrent infection.

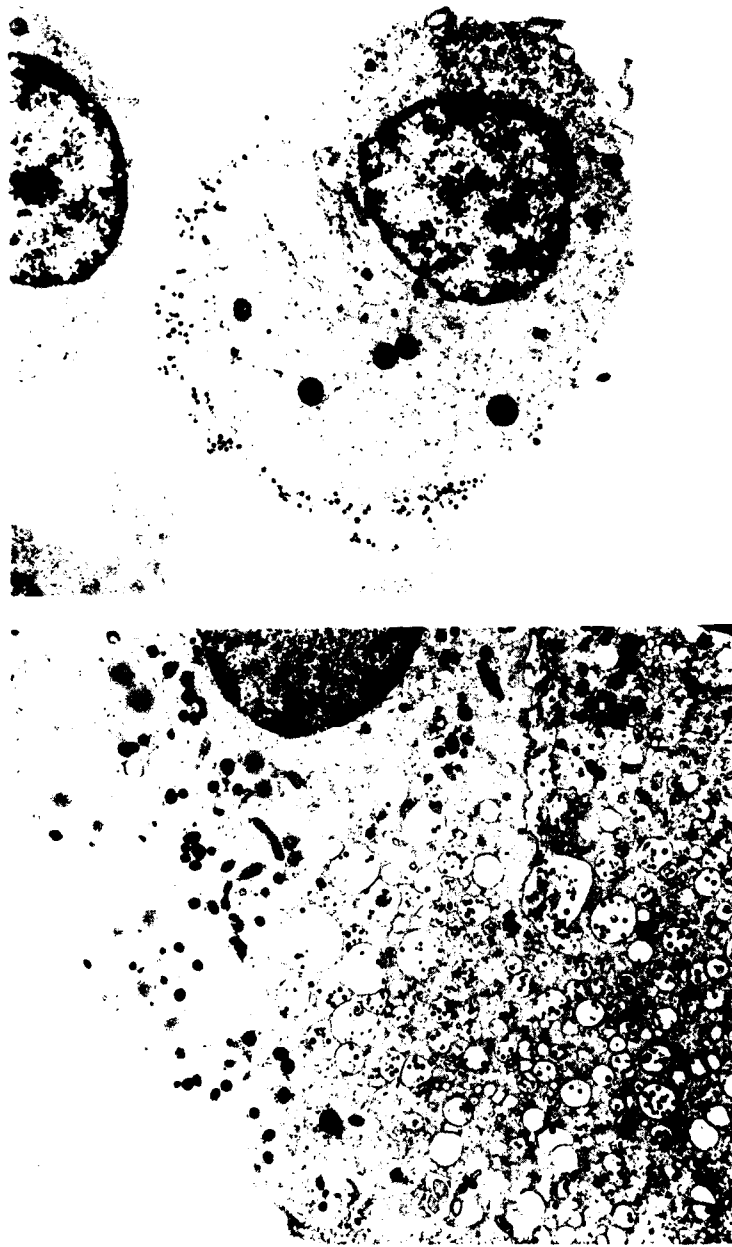


Figure 3 Virion budding and release in HIV-infected MCSF-treated monocytes and PHA/IL-2 treated lymphoblasts. Transmission electron microscopy of an HIV-infected MCSF-treated monocyte (*left*) (viral particles sequestered within intracytoplasmic vacuoles; few or no virions at the plasma membrane) and PHA/IL-2 treated lymphoblasts (*right*) (numerous viral particles budding at the plasma membrane; no intracellular virions), $\times 9400$ (51, 58). (The authors thank Dr. Jan M. Orenstein, Dept. of Pathology, George Washington University Medical Center, Washington, DC for electron microscopy.)

Mechanisms of HIV Entry Into Mononuclear Phagocytes

Analysis of HIV-infected macrophages by transmission electron microscopy suggests another fundamental difference between virus-macrophage and virus-T cell interactions. Mature virions released from T cells show characteristic surface projections or "spikes." Such "spikes" represent the gp120 envelope protein which is located entirely exterior to the virus membrane and is noncovalently associated with the gp41 anchor protein within the virus membrane. HIV that accumulate within vacuoles in macrophages are relatively bald (58)—that is, little or no gp120 is detected by such microscopic analysis. This relative lack of gp120 in the virions associated with HIV-infected monocytes was also evident in radioimmuno-precipitation analysis of viral protein synthesis (Figure 4). The predominant virus proteins synthesized by HIV-infected T cells are the envelope proteins. The virus encodes two envelope glycoproteins, gp120 and gp41, which are cleavage products of a precursor gp160 molecule. The external gp120 binds to T-cell-membrane CD4 as an initial and obligatory event in T-cell infection. After a 1 hr pulse of radiolabeling, envelope proteins and their breakdown products exceed that of capsid proteins (*gag* gene products) and their breakdown products. This ratio (*env* > *gag* gene products) is maintained for both T-cell synthesis of viral proteins and protein assembly into viral particles. In contrast, the dominant viral proteins synthesized in the HIV-infected macrophage and assembled into viral particles are capsid proteins. There is a relative deficiency of *env* gene products both in the infected cell and in the infectious virus. This relative lack of both virus gp120 and macrophage CD4 suggests the possibility for other mechanisms of virus entry.

We explored this possibility by studying the ability of soluble recombinant CD4 (sCD4) to competitively inhibit HIV infection of macrophages (P. J. Gomatos, N. M. Stamatou, H. E. Gendelman, A. Fowler, D. L. Hoover, D. C. Kalter, D. S. Burke, E. C. Tramont, M. S. Meltzer. "Lack of effect for soluble CD4 on infection of human monocytes by HIV-1." Submitted for publication). sCD4 binds with high affinity to gp120 ($K_D \sim 10^{-9}$ M) and at relatively low concentrations ($< 1 \mu\text{g/ml}$) inhibits the infection of T cells or T-cell lines by many HIV strains (59–62). Initial studies confirmed that HTLV-III_B infection of the H9 T-cell line was completely inhibited by prior treatment of virus with $10 \mu\text{g/ml}$ sCD4 and with the same concentration of sCD4 continuously maintained in culture. No p24 antigen was detected in culture fluids of H9 cells exposed to 10^5 TCID₅₀ HTLV-III_B with sCD4 through 10 days. HTLV-III_B infected H9 cells showed about 215 syncytia per 10^5 cells without CD4, but no syncytia were observed in replicate cultures treated with $10 \mu\text{g/ml}$ sCD4. In other

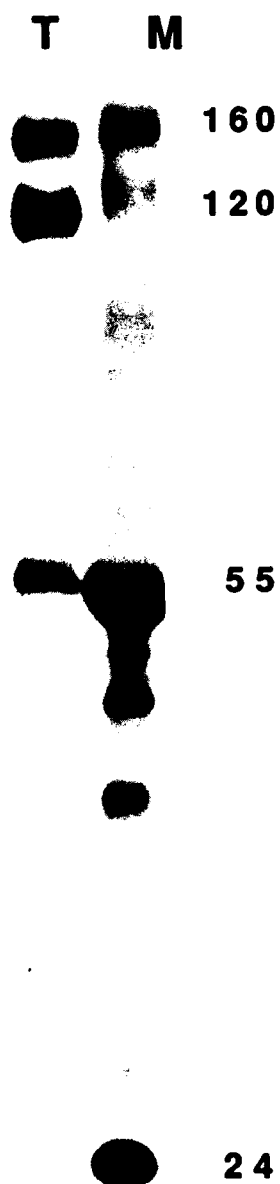


Figure 4 Viral protein synthesis in HIV-infected MCSF treated monocytes and PHA/IL-2 treated lymphoblasts. HIV-specific proteins shown after gel electrophoresis and autoradiography were isolated by radioimmunoprecipitation with pooled HIV-seropositive sera of ^{35}S -methionine labeled cell lysates from MCSF-treated monocytes (M) infected with ADA, a monocyte tropic HIV-1 isolate or PHA/IL-2 treated lymphoblasts (T) infected with HTLV-III_B.

experiments and as previously reported, HTLV-III_B infection of H9 cells was completely inhibited by as little as 0.1–1 $\mu\text{g/ml}$ sCD4. Under identical conditions, sCD4 at 10 $\mu\text{g ml}^{-1}$ had little or no effect on release of p24 antigen in culture fluids of MCSF-treated monocytes infected with 10² TCID₅₀ ADA, a monocyte tropic HIV patient isolate (51). It is noteworthy that the HTLV-III_B used for infection of H9 cells in these experiments contained > 100-fold more infectious particles than did the ADA inoculum used to infect monocytes. Thus, at least a 10,000-fold difference exists in the capacity of sCD4 to inhibit HIV infection in T cells, compared to macrophages.

The inability of sCD4 to inhibit HIV infection of macrophages was confirmed by two other independent means of detecting virus infection. Multinucleated giant cells were evident in about 20–40% of cells in the HIV-infected cultures. No difference appeared in number or extent of these multinucleated giant cells in replicate cultures treated with sCD4. Similarly, about 20–30% of total macrophages expressed HIV mRNA, as detected by in situ hybridization with radio-labeled, single-stranded RNA probes at 2 weeks. No differences were observed in the frequency of macrophages that express HIV mRNA with or without sCD4.

The inability of sCD4 to inhibit infection of HIV in macrophages was not limited to the ADA isolate. We examined six different HIV isolates that infect macrophages. HIV inoculum was less than 0.001 macrophage infectious doses per cell. In each instance, 10 $\mu\text{g ml}^{-1}$ sCD4 had little or no effect on virus infection. Levels of p24 antigen released into macrophage culture fluids at 3 weeks with or without sCD4 were indistinguishable; HIV-induced cytopathic effects were evident in both CD4-treated and untreated cultures.

The role of plasma membrane CD4 in the initiation of infection by HIV in macrophages was further examined by two different approaches to block these receptors: (a) competitive inhibition by excess HTLV-III_B, and HIV strain that interacts with CD4 receptors in many different cell lines but fails to infect macrophages, and (b) use of monoclonal antibodies that bind to epitopes on CD4 close to or at the site of binding for virus gp120. We exposed macrophages to HTLV-III_B at an inoculum titer 1000- to 10,000-fold more than that used for any of the monocyte tropic HIV isolates. The time course and ultimate levels of p24 antigen released into macrophage culture fluids after infection with any of the monocyte tropic HIV isolates were unchanged by prior exposure of these cells to an excess of HTLV-III_B. Thus, if HTLV-III_B occupied the CD4 receptors on the macrophages, such receptor occupancy did not affect the ability of monocyte tropic HIV isolates to initiate a productive infection.

These observations raise the possibility of another receptor or mech-

anism of entry for HIV in macrophages independent of CD4. However, this possibility was made less likely by subsequent experiments with monoclonal antibodies directed against the gp120 binding site on CD4. Infection of macrophages by monocyte-tropic HIV isolates was completely abrogated by prior treatment of cells with the monoclonal antibodies, Leu3a or OKT4a. As a control, equal amounts of monoclonal antibody directed against HLA-DR had no effect.

If CD4 is the receptor for HIV on macrophages, then (a) the interaction of sCD4 with monocyte tropic HIV may differ from that of T cell-tropic HIV (perhaps similar in concept to previously observed differences in efficacy of sCD4 for inhibition of HIV-1 and HIV-2 infection) (63), or (b) the interaction of sCD4 (which lacks the transmembrane and cytoplasmic sequences of CD4) with monocyte tropic HIV may be different from that of the CD4 on macrophage plasma membranes. Alternatively, some or all HIV receptor(s) on macrophages may be different from CD4: (a) HIV may enter macrophages through phagocytosis, FcR-mediated endocytosis (64-67), or interaction with receptors for mannosylated proteins (68), or (b) as the monocyte tropic HIV strains are relatively deficient in gp120 (as documented by radioimmunoprecipitation analysis of protein synthesis in HIV-infected macrophage lysates and by absence of envelope "spikes" observed by transmission electron microscopy), infection of macrophages may require direct interaction of gp41 with some membrane component to initiate virus-plasma membrane fusion (69). Binding of monoclonal anti-CD4 to macrophages may cover this putative fusion site; binding of HTLV-III_B may not. By whatever mechanism, the preceding data convincingly shows that sCD4 is much less effective in blocking infection of macrophages than of T cells. If the results obtained in cell culture systems have clinical significance, then macrophages which serve as reservoirs for HIV throughout the body, and which may ultimately be responsible for chronic CNS disease, will not be protected by sCD4 treatment.

Antibody-Dependent Enhancement

Several recent reports document at least one CD4-independent route of infection in susceptible T-cell and macrophage targets (64-67). Infection of T cells or macrophages with HIV was markedly enhanced (5- to 10-fold increase in reverse transcriptase activity in culture fluids) by sera from certain HIV-infected patients. The enhancement phenomenon in T cells was dependent upon either serum antibody or components of the alternative complement cascade (64). However, no role for the alternative complement cascade was documented for HIV infection of macrophages. Antibody-mediated enhancement in both macrophages and T cells was not inhibited by monoclonal anti-CD4 (Leu3a) or by sCD4 (67). Thus,

HIV can bind to and enter susceptible target cells without interaction with CD4. The binding site and mechanism for virus entry are not yet defined. In macrophages, antibody-mediated enhancement of HIV infection is inhibited by monoclonal anti-FcRIII (the predominant Fc receptor in tissue macrophages and monocytes in culture, which is absent on circulating blood monocytes), but not by monoclonal antibodies directed against FcRI or FcRII (67). Antibody-mediated enhancement of HIV infection in the myeloid cell line U937, which lacks FcRIII but does express FcRI and FcRII, was blocked by heat-aggregated IgG (66). Antibody-mediated enhancement in T cells, cells that lack FcRI, FcRII, and FcRIII, was not inhibited by monoclonal antibodies against FcRI, FcRII, or FcRIII, but was also inhibited by heat-aggregated IgG (67). Most impressively, strains of HIV that do not normally replicate in macrophages do so after antibody-mediated enhancement of infection, an observation suggesting that the basis for macrophage tropism may reside within the mechanisms for virus entry (65).

MONONUCLEAR PHAGOCYTES AS REGULATORY CELLS IN THE PATHOPHYSIOLOGY OF HIV INFECTION

The preceding observations clearly document major roles for macrophages as both target cell and reservoir for infectious virus during HIV disease. HIV-infected macrophages are found in brain, lung, lymph node, skin, bone marrow, and blood of seropositive patients. In certain tissues, notably brain and lymph nodes, the frequency of infected cells approaches 1 in 10. It is probable that these infected cells directly participate in the pathogenesis of HIV-induced immunosuppression and central nervous system dysfunction. However, the means and mediators for this participation are not yet understood. A major role for macrophages in the steady state and during disease is regulation of tissue function. This regulatory role is mediated by the literally hundreds of secretory molecules released by the macrophage under a variety of pathophysiologic conditions (70). Changes in the secretion or release of certain mediators occur during HIV infection and underlies the symptomatology of AIDS. For example, disordered secretion of the monokine $\text{TNF}\alpha$, or cachectin, has been postulated as the humoral basis for "slim disease," a wasting syndrome unrelated to opportunistic infection, commonly seen in African AIDS (71).

The paucity of virus-infected lymphocytes in AIDS and the absence of cytolytic infections of neurons or neuroglia suggest an indirect mechanism for immune and nervous system dysfunction in HIV infection. For example, macrophages release many secretory products that have direct

effects on nerve growth, function, or repair of injury. Inappropriate secretion of these monokines (IL-1, IL-6, TNF α , platelet-derived growth factor, apolipoprotein e) by HIV-infected macrophages in the brain may induce both neurologic symptoms and tissue injury. Moreover, macrophages have receptors for and respond to several neuropeptides (ACTH, β -endorphins, somatotropin, neurotensin, substance P, and vasoactive intestinal peptide) to secrete toxic oxygen metabolites and other injurious monokines. Indeed, recent reports document induction of prostaglandins IL-1 and IL-6 by gp120, the HIV-envelope protein (30, 31). Furthermore, a highly conserved region of gp120 is partially homologous to neuroleukin, a cytokine with neurotrophic activity. Addition of gp120 to neurons in vitro inhibits the neurotrophic activity of neuroleukin (72). These individual observations provide the basis for a regulatory network or loop in which HIV-infected macrophages affect nerve cells through any of several monokine or virus-derived secretory factors; the injured neural tissue in turn prompts the HIV-infected macrophage to release even more toxic secretory products (73).

There is increasing evidence that such a regulatory loop also regulates virus production and latency in HIV-infected macrophages and T cells. HIV subverts cellular transcriptional factors and other cellular gene products to direct virus gene expression. The HIV long terminal repeat (LTR) contains a number of *cis*-acting sequences that are targets for trans-activators, including the cellular DNA-binding proteins NF- κ B and Sp1 (74, 75). Activation of the HIV LTR in proliferating T cells after treatment with mitogens or phorbol esters is associated with synthesis of the endogenous DNA-binding protein, NF- κ B (74). NF- κ B binding activity is also present in normal human monocytes and macrophages. HIV gene expression in the myeloid cell line U937 is regulated by NF- κ B: treatments that induce NF- κ B binding activity in U937 (phorbol esters, TNF α) markedly increase HIV replication (76). But the HIV LTR can also be activated by endogenous signals independent of NF- κ B. GM-CSF which does not induce NF- κ B binding activity also activates HIV expression in U937 (77-79).

An animal model that simulates latent HIV infection in humans was produced to further identify factors that affect induction of viral gene synthesis in latently infected target cells. Transgenic mice that contain integrated copies of the HIV LTR linked to the bacterial gene for chloramphenicol acetyltransferase (CAT) were constructed (80). The LTR contains all known HIV transcriptional signals. Thus, HIV LTR-directed expression of the CAT gene in cells of transgenic animals would be analogous to HIV-gene expression for progeny virus production in infected patients whose cells harbor latent integrated proviruses. Under steady-

state conditions in blood and in tissue, neither macrophages nor lymphocytes expressed detectable levels of CAT activity. CAT activity in macrophage populations was increased by *in vitro* treatment with any of several recombinant murine cytokines (IL-1, IL-2, IL-4, MCSF, and GMCSF). A similar phenomenon was observed *in vivo*. Resident peritoneal macrophages or cells elicited by a sterile, chronic irritant (thioglycollate) showed no CAT activity. However, macrophages recovered from an intraperitoneal immune reaction to *Mycobacterium bovis*, strain BCG or *Propionibacterium acnes* (*Corynebacterium parvum*) were strongly positive. Purified splenic T cells also showed no constitutive CAT activity. Significantly, cultivation of these T cells in murine IL-2 increased CAT expression 20-fold. Infection of both macrophages and T cells with certain DNA viruses (herpes simplex 1, adenovirus, murine cytomegalovirus) increased CAT activity by as much as 50-fold. Thus, HIV activation in this mouse model system is clearly regulated by endogenous cytokines released from both T cells and macrophages.

The long interval of clinical latency during HIV infection (50% of infected individuals develop AIDS within 10 yr) (1) may be associated with true viral latency (perhaps intermittent) with no expression of HIV in infected cells. In the latent state, HIV exists as a provirus integrated within host genomic DNA without transcriptional activity. HIV becomes transcriptionally active in T cells and reenters the replicative cycle after exposure of the cell to a variety of apparently unrelated stimuli that include mitogens, phorbol esters, infection with herpesvirus, adenovirus, and exposure to sunlight. Cellular regulatory elements can both increase and decrease HIV activation. In T cells, the mitogen-induced protein, HIVEN86A binds to HIV LTR enhancer elements and the IL-2 receptor gene (81). In resting T cells, the regulatory protein rpt-1 downregulates HIV-LTR activity and IL-2 receptor gene expression (82). Monocytes exposed to bacterial endotoxic lipopolysaccharides release IL-1 and TNF α , which in turn increases HIV expression in T cells (83). TNF α induces DNA binding proteins that activate the HIV LTR by binding to NF- κ B sites in T cells and macrophages. Monokines also induce cytokine secretion from T cells. The lymphokines GMCSF and IL-3 both increase HIV expression in monocytes (79, 84). Thus, as with the macrophage-neural tissue interactions, another bidirectional regulatory loop is formed to regulate HIV expression in macrophages and T cells.

SUMMARY

We have presented evidence in this review for the following: (a) Macrophages are likely the first cell infected by HIV. Recovery of HIV from

macrophages has been documented in the early stages of infection in which virus isolation in T cells is unsuccessful and detectable levels of antibodies against HIV are absent. (b) Macrophages are major reservoirs for HIV during all stages of infection. Unlike the lytic infection of T cells, HIV-infected macrophages show little or no virus-induced cytopathic effects. HIV-infected macrophages persist in tissue for extended periods of time (months) with large numbers of infectious particles contained within intracytoplasmic vacuoles. (c) Macrophages are a vector for the spread of infection to different tissues within the patient and between individuals. Several studies suggest a "Trojan horse" role for HIV-infected macrophages in the dissemination of infectious particles. The predominant cell in most bodily fluids (alveolar fluid, colostrum, semen, vaginal secretions) is the macrophage. In semen, for example, the numbers of macrophages exceed those of lymphocytes by more than 20-fold. (d) Macrophages are major regulatory cells that control the pace and intensity of disease progression in HIV infection. Macrophage secretory products are implicated in the pathogenesis of CNS disease and in control of viral latency in HIV-infected T cells. This litany of events in which macrophages participate in HIV-infection in humans parallels similar observations in such animal lentivirus infections as visna-maedi or caprine arthritis-encephalitis viruses. HIV interacts with monocytes differently than with T cells. Understanding this interaction may more clearly define both the pathogenesis of HIV disease and strategies for therapeutic intervention.

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